

THE PHYSIOLOGICAL BASIS OF WING PRODUCTION IN THE GRAIN APHID

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TWO CHARTS

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¹ Contribution from the Zoölogical Laboratory of the University of Michigan.

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INTRODUCTORY

Of the ten to seventy or more sisters that are produced parthenogenetically by a single aphid, all may be winged, or all may be wingless, or both winged and wingless forms may occur in varying proportions. Several other morphological and physiological variations also occur. These other variations are strongly correlated with the presence and absence of wings and occur in such a way as to make it apparent that whatever may be the basis of wing production is also the cause of certain other variations that occur in parthenogenetic lines of aphids.

Many investigators have sought to account for the variations in wing production as they occur in a parthenogenetic line of aphids. In most cases, however, aside from occasional suggestions of a physiological or genetic nature, they have concerned themselves primarily with the strictly ecological aspects of the problem. The data obtained have been frequently contradictory or inconclusive. This is particularly true with reference to the effects of temperature and of various salts on wing production. Investigations into the effects of starvation, however, have given fairly uniform results, and all the data so far collected indicate that winged parents are less likely to have winged offspring than are wingless parents.

In addition to investigating the effects of environmental factors on wing production under more rigid conditions than have been heretofore observed, a study was made of the variations correlated with wing production. The contents of the body cavity were also subjected to a careful examination. New facts have been brought to light with respect to the

globules contained therein, which are believed to have an important bearing on the problem of wing production. An effort is made in this paper to associate all the facts that are concerned with the development of wings and to interpret these facts upon a physiological basis.

To Prof. A. Franklin Shull, under whose helpful supervision this work was done, and to those other members of the faculty of the University of Michigan who have been of assistance, I wish to express my sincere appreciation.

METHODS AND APPARATUS

All of the aphids used in these experiments were the offspring of a single female, the progenitors of which had been growing in the laboratory under uncontrolled conditions for approximately two months. They were identified by Mr. C. W. Mason, of the Bureau of Entomology, Washington, as *Rhopalosiphum prunifoliae* Fitch, a species frequently mistaken for *Aphis avenae* L.

The oat seedlings on which the aphids were reared were germinated in sand moistened with nutrient salt solution. After germination, the seedlings were grown in quart Ball-Mason jars, the outsides of which were covered with black paper and around the shoulders of which were glued strips of soft felt-like cloth. Lantern chimneys were placed over the Mason jars, their larger ends resting on the cloth-covered shoulders. The upper ends of the lantern chimneys were covered with pieces of thin cloth. The Mason jars were filled with a nutrient salt solution which will shortly be described. A paraffin float, about $\frac{1}{4}$ -inch thick and having a diameter slightly smaller than the neck of the jar, was placed in the solution. These floats each had six or seven small holes for the reception of the seedlings.

Throughout the temperature and type-of-parent experiments and in all other cases where different conditions are not specifically described, the aphids were reared on plants growing in a solution which was made up as follows from

stock solutions A and B. Solution A contained 10 grams of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and ten drops of a 1 per cent colloidal solution of FePO_4 to a liter of water. Solution B consisted of 6 grams KH_2PO_4 , 12 grams $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 7.5 grams KNO_3 to 1 liter of water. The nutrient salt solution was made up in the following proportions: 75 cc. solution A, 75 cc. solution B, and 1 cc. of $n/2 \text{Na}_2\text{CO}_3$ to 849 cc. of distilled water. This gave a solution having a pH value of approximately 6.0, which is nearly the same as that found by Salter and McIlvaine ('20) to be the optimum H-ion concentration for the growth of wheat seedlings. Aside from the Na_2CO_3 , which was added for the purpose of controlling the H-ion concentration, the solution is of nearly the same composition as Tottingham's ('14) solution of medium concentration.

A single oat seedling which had been growing in this kind of solution for four days after germination was placed in another Mason jar containing a fresh solution, and a single aphid which was always of the wingless type, unless otherwise stated, was placed on the plant. Fresh plants were provided for the parent at least every second day and sometimes every day at the higher temperatures, but less frequently at low temperatures. These fresh plants were placed in the same jar as the first plant, and thus all the offspring of a single parent were raised in the same jar, uniform food was provided, and overcrowding was avoided. The young were removed and recorded as soon as their nature with respect to wing production could be determined with certainty.

The aphids, and the seedlings on which they were reared, were grown in constant-temperature chambers. These chambers, four in number, were each large enough to accommodate twelve jars. The air of each chamber was kept in circulation with an electric fan. Large glass doors permitted the entrance of adequate direct sunlight. Thermographs were kept constantly in two of the four chambers.

The apparatus was capable of maintaining a temperature that usually exhibited only minor variations of one degree or less in all used portions of the chambers. Accidents to the cooling apparatus, to the heating units, or the thermostatic mechanism occurred occasionally, but would usually be corrected within a few hours.

THE EFFECTS OF TEMPERATURE ON WING PRODUCTION

Specific temperatures

Ewing ('16), while studying the effects of selection on a parthenogenetic line of *Aphis avenae* (= *Rhopalosiphum prunifoliae*?), made numerous keen observations on the general biology of aphids. It appears that his paper contains the first evidence to be derived from experimentation which indicates that winged forms of aphids are more likely to be produced at certain temperatures than at other temperatures. He found that of forty-four aphids raised at 60°F. (16° + C.) all were winged; of thirty-seven aphids raised at 70°F. (about 21°C.) only 15.1 per cent were winged, and of the sixty-one aphids raised at 80°F. (about 27°C.) 69.6 per cent were winged. Wadley ('23) raised much larger numbers of aphids and secured results which also indicated that winged forms are more likely to be produced at certain temperatures than at other temperatures, but the percentages and the differences he secured were so small as to leave some doubt as to their significance.

Call ('17-'18) found that no winged forms of *Aphis maidis* developed when reared at 84° to 90°F.; only one was winged out of several hundred at 72°F., and when raised at 60° to 70°F. large numbers of winged forms appeared. No moderately high temperatures were included in Call's experiments. In 1918, Shinji studied the effects of various salts on wing production with a number of different aphids. Although he carried out no adequate experiments to determine the effects of temperature, he states his belief that "these observations, establish the fact, that

changes in temperature have no effect on the production of winged forms.”

To determine the effects of temperature on the grain aphid, the insects were reared on plants growing in salt solutions as previously described. These aphids were raised at temperatures of 14°, 16°, 18°, 20°, 24°, and 26°. The first-generation aphids at 16°C. were obtained from the 26° chamber; the first generation aphids at 14°, 20°, and 26° were obtained from parents that were born at 16°. The 24° parents of the first generation were obtained from the 20° chamber, and the 18° parents from the 10°, 14°, 24°, and 26° chambers. The percentages of wing production in this last case were too low to show any significant differences in the percentages obtained from aphids which were derived from the different sources.

For the 14°, 18°, 20°, and 24° boxes the temperature fluctuations that extended beyond one degree were few in number and were usually due to accidents to the mechanical equipment, which were rectified within a few hours, and probably had but little effect on the final results. Somewhat wider variations occurred during the progress of the 16° and 26° experiments, and the high percentages obtained for these temperatures may be due in part to such irregularities.

During the first generation at 16°C., as shown in table 1 and graph 1, thirty-two of the eighty-one aphids raised, or 39.5 per cent, were winged. In succeeding generations at this temperature, the parent in each case having been raised at 16°C., 239 of the 696 aphids, or 34.4 per cent, were winged. As may be seen in the accompanying graph and tables, aphids that were raised at 18° and 20° and at 12° (the 12° results are from a second series of experiments) were less likely to be winged than those raised at either 14° to 16° or 24° to 26°. In other words, there are two points of high wing production (16° and 24° to 26°) and two points of low wing production (12° and 18° to 20°).

From the few cases where aphids were grown at 28°C. it appeared that the percentage of winged forms produced might

show a decrease rather than a further increase. It was considered inadvisable, however, to secure data from aphids grown at temperature higher than 26°C., because of the harmful effect of the high temperatures on the oat seedlings and on the general vitality of the aphid.

For the purpose of checking up some of these results, a second series of experiments was conducted under similar conditions during October and November at temperatures of 12°, 16°, and 18°. The first-generation parents in each case were obtained from a line that had been growing at 24° for several months. The temperature at 16° and 18° underwent only minor variations. At 12° the temperature would

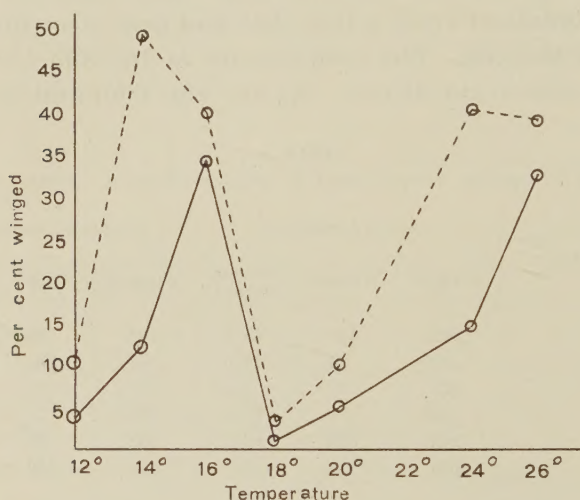
TABLE 1
Relation of specific temperatures to wing production. First series

TEMPERATURE	FIRST GENERATION			SUCCEEDING GENERATIONS		
	Wingless	Winged	Per cent winged	Wingless	Winged	Per cent winged
14°	242	230	48.7	448	63	12.3
16°	49	32	39.5	457	239	34.4
18°	519	20	3.7	237	3	1.2
20°	98	11	10.1	473	28	5.6
24°	295	196	40	348	60	14.7
26°	103	65	38.7	313	149	32.4

rise a few degrees or so at midday, owing to the heat of the sun, and occasionally at other times. The main features of these results as given in table 2 are in agreement with those given in table 1; that is, a higher percentage of winged forms was produced at 16° than at lower temperatures, and a much higher percentage than at 18°.

It is appreciated that all of the above data are readily affected by the degree of overcrowding, the age of the plant, and probably other factors, so that even though the greatest care is taken to control all factors affecting wing production in a uniform manner, it is well nigh impossible to do so for all temperatures throughout the long period of time necessary for the accumulation of such data. To these factors

are doubtless due the minor irregularities in the wing-production curves and the discrepancies between different experiments designed to test the effects of temperature. Nevertheless, there can be little doubt that certain ranges of temperature are more likely to produce winged forms than other ranges of temperature, and that there are two points of high wing production (16° and 24° to 26°) and two points of low wing production (12° and 18° to 20°).



Graph 1 The percentage of winged forms appearing at various temperatures. — — — —, first generation; —————, succeeding generations. The values for 12° are taken from the second series of temperature experiments.

What the nature of this temperature effect is, no one has endeavored to determine. Because of the similarity between the curve shown in graph 1 and that of Heilbrunn ('24) for the effect of temperature on the viscosity of protoplasm of *Cumingia* eggs and because of the results obtained by Shinji in which he found the salts of magnesium and of the heavy metals to be 'wing-developing' substances, as opposed to the salts of the alkalies and alkaline earths (Ca and Sr) which he found to be 'non-wing-developing' substances, it was at first thought that wing production might be related to

changes in the viscosity of protoplasm. Inasmuch as I have been unable to confirm Shinji's results, however, the idea has not been pursued further, but is by no means disproved.

Graph 1 makes it appear as though, whatever the nature of the influence of temperature may be, some sort of an interfering factor enters in between 16° and 18°. It will be shown later that certain changes in the pigmented globules contained in the haemolymph occur with changes of temperature, especially at 16°C. A further discussion of these relationships will be deferred to later sections.

TABLE 2

Relation of specific temperatures to wing production. Second series

TEMPERATURE	FIRST GENERATION			SUCCEEDING GENERATIONS		
	Wingless	Winged	Per cent winged	Wingless	Winged	Per cent winged
12°	179	21	10.9	69	3	4.2
16°	158	75	32.2	68	5	6.9
18°	345	6	1.7	111	1	0.9

Constancy of temperature

In all cases when aphids were changed from chambers in which few or no winged forms were being produced to another temperature, the first offspring to appear were usually wingless. After a period of three to ten days, winged forms appeared in increasing proportions, the maximum being reached in from one to two weeks. This period was followed by a gradual decline in wing production ending in the production of few or no winged forms. The period of maximum wing production coincided rather closely with the close of the first generation.

A typical condition is represented by aphids raised at 24°C. Of the 107 aphids recorded during the first seven-day period, only 9.3 per cent were winged, while during the second period of seven days, 48 per cent of the 332 aphids recorded were winged. For the third period of seven days, 112 aphids

were recorded, of which 35.7 per cent were winged; the fourth period yielded 77 aphids, of which 9 per cent were winged; the fifth period, 184 aphids, of which 20 per cent were winged, and of the last 87 aphids only 3.3 per cent were winged. This line was continued at 24°, but under varying conditions of overcrowding and on plants in various salt solutions. After a period of two months, the conditions which prevailed throughout the temperature experiments were restored, and 280 more aphids were raised over a period of six weeks, all of which were wingless. The results are shown in graph 2.

For all temperatures the last families of aphids raised show a marked reduction in the percentage of winged offspring when compared with the percentages given for first-generation aphids in table 1. Thus of the 229 offspring of the last eight families of aphids raised at 26°C. only 0.44 per cent were winged; of the 280 offspring from the last ten families at 20°C. only 0.7 per cent were winged; of the 270 offspring from the ten last families at 18°C. none were winged, and of the 147 offspring from the six last families raised at 16°C. only six aphids, or 4 per cent, were winged.

It appears also that constancy of temperature affects the number of offspring born. During the first generation at 18°C., 539 offspring were recorded as being born of 12 parents, or an average of 45 aphids per parent. During the second generation at this temperature, the average number of offspring was only 20, a total of 240 offspring having been born to 12 parents. At 24°C., ten parents in the first generation yielded 491 offspring, an average of 49.1 aphids per parent, while in the second generation 12 parents bore 384 young, or an average of 32 per parent. It should be pointed out, however, that no special effort was made to record every aphid born. In all cases some aphids undoubtedly died or migrated from the plants and thus were not recorded.

These data show that when aphids are changed from one temperature to another, a higher percentage of winged forms is produced during the first two or three weeks after the change than later on, but that a period of three to ten days

must elapse before the change of temperature effects wing production. It also appears that a general increase of vitality accompanies changes of temperature.

No previous studies on wing production in aphids refer to the effect of maintaining aphids at a constant temperature. Ewing ('16), however, during his study of inheritance in aphids, made numerous measurements of body length and found that the mean length of aphids raised at constant temperatures was 'far below' the mean of his pure-line stock. He asks, "Can it be that constant temperatures no matter of what degree, have a dwarfing effect on plant lice? Does the changing of temperature stimulate growth and metabolism in general?"



Graph 2 Distribution of winged forms over a period of several months at 24°C., showing rapid increase of wing production after the first few days and a gradual decline to zero thereafter.

With other organisms, Issakowitsch ('05) found that the longer daphnids were kept at a temperature of 24°C. the stronger was the tendency for them to produce only parthenogenetic females. He thinks, however, that temperature affected the organism only indirectly through changing its food supply. Matisse ('19) found that when certain snails, crabs, and worms were changed from one temperature to another and then kept at the latter temperature constantly, their activity was lowered at first quite rapidly and after two or three days very slowly. He associated the whole phenomenon with changes in the size and number of ultramicroscopic pro-

toplasmic granules in their relations to the availability of food.

Were these the only data, there might well be still some doubt as to whether aphids (and certain other organisms) are directly affected by constancy of temperature conditions and not merely indirectly through the effect of temperature changes on the food of the organism. It will be shown, however, that aphids kept at a given temperature for a sufficient length of time arrive at an equilibrium with their thermal environment. This process can be followed day by day until it reaches completion. It will be shown that for each habitat temperature the fat-globules of aphids have a specific solidification temperature. Further, it will be shown that when aphids are changed from one habitat temperature to another, a generation or more must usually intervene before the specific fat-solidification point of the new thermal environment has been reached by all of the fat-globules. It is believed that there is sufficient evidence to show that, in so far as these fat-globules are concerned, temperature conditions primarily have a direct effect on the aphid, and not merely indirectly through its food.

An interpretation of the changes which occur in the fat-solidification temperatures in their relations to the time (three to ten days) that elapses after aphids are transferred to a new temperature before an effect is apparent in the percentages of winged forms produced is deferred to the section given over to general discussion.

Conclusions concerning temperatures

1) Certain ranges of temperature are more likely to produce winged forms than other ranges of temperature, there being two points for high wing production (16° and 24° to 26°) and two points for low wing production (12° and 18° to 20°). 2) When aphids that have been growing at a particular temperature are transferred to a new temperature, they produce a higher percentage of winged forms during the first two or three weeks than they do later on. 3) A period

of three to ten days must elapse before the effects of temperature changes on wing production become effective. 4) A change of temperature appears to stimulate growth and metabolism in general.

TYPE OF PARENT

The results of other investigations have so uniformly indicated that winged parents are less likely than wingless parents to give birth to winged offspring that only a few settings of winged parents were made. Observations by both Klodnitski ('12) and Miss Gregory ('17) indicated that winged parents bear a smaller proportion of winged offspring than do wingless parents, but it remained for Shull ('18) to carry out experiments specifically designed to prove this point. He found that winged parents were not only less likely to produce winged offspring than were wingless parents, but also that in the sexual part of the cycle winged viviparous females produce mostly sexual females, while the wingless viviparous females produce mostly males. Similar results with respect to the parthenogenetic portion of the cycle were obtained by Davidson ('21 a, b) and Wadley ('23). In *Lachnus pini*, Mason ('22) observed that a more or less perfect alternation of winged forms occurred, the majority of the offspring from each kind of parent belonging to the opposite type.

The offspring of winged parents were reared under the same conditions as were the offspring of wingless parents during the progress of the temperature experiments. The one thus acted as a check on the other. Of 408 offspring from 10 winged parents raised at 26°C. only 5.1 per cent were winged as compared with 32 per cent when wingless parents were used. Of 126 aphids raised from winged parents during their first generation at 24°C. only 5.2 per cent were winged as compared with 40 per cent for the wingless parents. During the second generation at 24° only 2.6 per cent of the 114 offspring from winged parents were winged as compared with 15.6 per cent for wingless parents of the second generation,

and at 14°C. 1.3 per cent of the 75 aphids born of winged parents possessed wings as compared with 19.9 per cent for the offspring of wingless parents of the second generation.

It thus appears that winged parents are less likely than wingless parents to have winged offspring.

Discussion for type-of-parent experiments

It was suggested to me early in this study that winged forms are less likely to be produced by winged parents than by wingless parents, because some substance necessary to the production of wings is consumed to such an extent during the development of the winged parent as to leave an amount that is insufficient for the production of winged offspring. Certain facts discovered later will be presented in connection with the starvation experiments, and also in the sections dealing with the brown globules that occur in the body cavity, that appear to support this contention.

STARVATION

Probably no single factor affecting wing production has been commented on so extensively as has starvation. Although all experimental work has yielded rather uniform and usually very striking evidence to support the contention that wing production is induced in part by starvation, nevertheless numerous opinions of an opposite nature have been expressed.

From breeding experiments Morgan ('85) and Keller ('87) concluded that a lack of food induced the appearance of winged forms in *Phylloxera vastatrix*. Miss Gregory ('17) studied the effects of starvation on three different sets of green-pea aphids while at New York (Columbia), Woods Hole, and Princeton, Massachusetts. In both the first and the last cases the starved parents produced about three times as high a percentage of winged forms as did the unstarved controls. In her Woods Hole experiments, however, for some unaccountable reason, none of the 188 offspring of starved

parents were winged. The experiments of Wadley ('23) similarly showed a striking increase in the percentage of winged offspring obtained from parents that had been starved.

From observations made during rearing experiments, which, however, were not specifically designed to determine the effects of starvation, Mordwilko ('07) and Davis ('09) infer that insufficient food induces the production of wings. Davidson ('14, '21 a) observed that a much higher percentage of winged forms appeared on plants that were heavily infested, but in his 1921 paper he concludes that this "is not necessarily connected with the condition of the plant, but due to the innate tendency of the apterous females to produce winged forms."

During their life-history studies on aphids, Baker and Turner ('16, '19) observed that, although the food supply was sometimes so poor as to make it difficult to maintain the insects, no unusual number of winged aphids appeared and in many cases no winged forms at all developed. Possibly the poor food conditions to which Baker and Turner refer are those resulting from a lack of sunlight on the plant rather than from being heavily infested with aphids. Tannreuther ('07), in an article on the germ cells of aphids, expresses the opinion that the production of winged forms is induced by an abundance of food rather than by a lack of it.

The contradictions that appear in the above comments may be due in part to the fact that starvation of the winged parent does not have the same effect as does starvation of the wingless parent. Miss Gregory ('17) found that when she starved winged parents the percentage of winged offspring underwent a slight decrease rather than an increase. Wadley ('23) secured similar results with winged parents under such conditions. In both cases only a small number of aphids were reared. Davidson's ('21 a, b) results also indicate that the starvation of winged parents does not favor the production of winged offspring.

Experiments with starvation of wingless parents

The effects of insufficient food in my experiments was determined by three methods: 1) Large numbers of aphids were allowed to grow on each plant, thus causing overcrowding and excessive injury to the plant. 2) Parents were removed from the food plants and placed in cotton-stoppered glass vials at 24° for a period of fifteen or sixteen hours and then raised under the same conditions as in the temperature experiments. 3) A line of aphids was maintained on plants that had been grown in the absence of sunlight and were so unfit for the growth of the insects as to make it difficult to maintain the line.

In all cases the aphids were reared at 24°C. and for the most part simultaneously with the end of the unstarved 24° line described in connection with the effect of temperature.

1. When two or three parents were placed on each plant in certain of the jars in the 24° chamber and allowed to remain on the plant so that their offspring were overcrowded, it was noted that a considerable number of the young so obtained were winged. Of 776 aphids so raised, 266 aphids, or 34 per cent, were winged in contrast with the entire lack of winged forms among the 280 aphids which were raised simultaneously under identical conditions, except that they were not permitted to overcrowd.

2. Nine wingless aphids were placed in cotton-stoppered glass vials for a period of fifteen or sixteen hours at 24°C. and their offspring were reared under the same conditions as in the temperature experiments. Of the 153 aphids so raised, 90 aphids, or 58.8 per cent, were winged. As may be seen in table 3, winged forms appeared consistently in each of the nine settings.

3. A line of aphids was reared on plants that had been grown in semidarkness for a period of four days after germination. The wingless parents were then placed on the plants and the jars with the plants and aphids were placed in a shaded portion of the 24° chambers. Because of the poor

condition of the plants, it was difficult to maintain the line after the first generation. Of the eighty-nine aphids raised under these conditions all were wingless. It is noteworthy that, although it was difficult to maintain these aphids because of poor food conditions, they did not show any marked reduction in size such as is so noticeable among aphids that are overcrowded.

TABLE 3
Effect of starvation on wing production at 24°C.

	PROGENY OF WINGLESS PARENTS			PROGENY OF WINGED PARENTS		
	Wingless	Winged	Per cent winged	Wingless	Winged	Per cent winged
Aphids overcrowded.....	510	266	34	175	11	6
No overcrowding.....	280	0	0	105	3	2.77
Parents removed 15 to 16 hours	4	4		204	3	1.4
	11	11				
	3	11				
	9	9				
	4	5				
	0	4				
	8	10				
	14	16				
	10	20				
Total.....	63	90	58.8			
Parents not removed.....	280	0	0	105	3	2.77
Plants weakened by lack of sunlight.....	163	0	0			

At various other times aphids were placed on plants that were so weak because of a lack of sunlight as to make it difficult to raise more than three or four aphids from a single parent. Of the seventy-four aphids so raised all were wingless.

Starvation of winged parents

It was found that when winged parents were starved the percentage of wing production in the offspring underwent a decrease rather than an increase. Winged parents were

placed in a glass vial at a temperature of 24° for a period of fifteen or sixteen hours. The parents were then placed on plants in the 24° chambers and grown under the conditions observed during the temperature experiments. Of the 207 offspring only three aphids, or 1.4 per cent, were winged. By comparing this percentage with the 2.77 per cent of winged forms obtained in the check experiment from unstarved winged parents and the 58.8 per cent obtained when wingless parents were similarly starved, it will be seen that the starvation of winged parents did not increase the percentage of winged forms produced and did not have the same effect as when wingless parents were starved.

The offspring of winged parents were allowed to live under overcrowded conditions and the percentage of winged forms produced was found to be slightly higher than when overcrowding was not permitted, as shown by the following test. From three to five winged parents were placed on a single plant and all of their offspring were born and reared on the same plant. Of 186 aphids so raised, eleven aphids, or 6 per cent, were winged. This percentage is much lower than the 34 per cent which was obtained when the offspring of wingless parents were raised under similar conditions, but is higher than the 2.77 per cent obtained from winged parents the young of which were not allowed to overcrowd.

Conclusions for starvation experiments

A shortage of food, if such shortage is due to either overcrowding or periodic starvation, results in a marked increase in the proportion of winged offspring produced by wingless parents. The offspring of wingless aphids that are grown on poorly lighted plants, however, show a decrease rather than an increase in the percentage of winged forms produced, even though the insects are so weak as a result of poor food conditions as to make it difficult to maintain the line.

Winged parents, if starved by periodic removal from the plant, show a decrease rather than an increase in the percentage of winged forms produced. A small increase in wing

production results if the offspring of winged parents are allowed to overcrowd, but the effect is much smaller than when the offspring of wingless parents are similarly treated.

Discussion of starvation results

A consideration of facts yet to be described has caused me to believe that wing production in these aphids may be dependent upon the concentration or proportion of certain substances in the haemolymph. How an increase in the concentration or proportion of these substances may be effected by internal changes resulting from starvation is discussed in a later section.

For the present I shall refer primarily to changes in the general metabolism of the plant which result from overcrowding and poor lighting, in so far as such changes may affect wing production.

Davidson ('14) believes that plants which are heavily infested cause aphids to become smaller and winged because of changes in either the quantity or quality of the sap. Ewing ('16) suggests that aphids grown on old plants are small (they are also more likely to be winged), because the aphid is unable to extract as much food from the harder tissues of older plants or because of changes in the nature of the plant juices.

In both cases where high wing production and smallness of size occur (this smallness of size refers to the wingless as well as the winged aphids), that is, on old plants and on heavily infested plants, the proportion of water present in plants generally is smaller than in young plants or in plants free from aphids. Davidson ('21), for instance, comments on the 'woody' nature of heavily infested *Euonymus* plants. It is presumed that woodiness implies a comparative deficiency of water.

In poorly lighted plants, however, one would expect a comparative abundance of water because of the decrease in photosynthetic activity resulting in a deficiency of carbohydrates. The food of aphids reared on such plants would be likely to

consist of a larger proportion of water and a smaller proportion of energy-yielding substances than if they were raised on well-lighted plants. Aphids reared on plants that are poorly lighted show neither an increase in wing production nor a reduction in size such as accompanies overcrowding, in spite of the fact that they barely receive sufficient food to permit their survival.

Both winged and wingless aphids raised at 24° vary in length from approximately 1 to over 2 mm., according to the conditions under which they are raised. Are aphids raised under overcrowded conditions and on older plants smaller because of a lack of water, and winged because of an increase in the concentration of certain substances in the haemolymph resulting in part from this same lack of water? Are aphids raised on poorly lighted plants and on freshly germinated plants wingless in spite of a lack of food, because of the comparative abundance of water?

These observations supported by the generally recognized fact that the wilting of plants due to excessive transpiration induces the production of winged forms (Woodworth, '08), make it appear as though the proportion of water taken in with the food of aphids may be one of the more important factors involved in wing production.

With winged parents, however, it is clear that periodic starvation does not tend to increase the proportion of winged offspring. Why does this contrast exist between winged and wingless parents? It seems reasonable to assume that a substance is present in wingless parents that is present to only a limited extent in winged parents (see the sections on type of parent and on coloration). It appears that the effective concentration or proportion of this substance may be increased as a result of starvation in the case of wingless parents (see the section given over to general discussion), while in the case of winged parents the substance is present in insufficient quantity to be affected by periodic starvation.

SALTS

According to Neills ('12), Loeb's studies of the influence of magnesium salts on living organisms suggested to Clarke ('03) that aphids also might show a differential response to various salts. He grew the rose aphid (*Nectarophora rosae*) on rose cuttings that had been placed in sand moistened with solutions of MgCl_2 , MgSO_4 , K_3PO_4 , and NaH_2PO_4 , and with distilled water. From 73 per cent to 92 per cent of the aphids raised on cuttings treated with magnesium salts were winged; only 2 per cent were winged when K_3PO_4 was used, none were winged with NaH_2PO_4 , and approximately 3 per cent were winged when water was used. Using MgSO_4 and following the same procedure with the same species of aphid as Clarke, Neills ('12) reared a small number of aphids that gave similar results.

Using methods similar to those of Clarke and Neills, but with certain other aphids in addition to the rose aphid, Shinji ('18) found that not only magnesium salts, but also all of the salts of the seven heavy metals that he used, as well as sugar and alcohol, caused a very marked increase in the proportion of winged forms produced. Moreover, when solutions of alum, acetic acid, tannin, urea, peptone, and of Na, K, Ca, and Sr salts were used in a similar manner, only a very small proportion of the aphids secured were winged. Shinji's results are very striking. With MgSO_4 solution, for instance, 831 out of 840 aphids were winged, while with sodium salts (?) only two out of 1029 were winged.

Unfortunately, Shinji gives no account of the previous history of the aphids which he used as parents, and only an inadequate account of parentage and starvation conditions. He merely states that "one to three viviparous females, usually apterous were planted," and that he had difficulty in keeping the aphids on the plants when certain salts were used, because of their poisonous nature.

Miss Haviland ('21) endeavored to increase the proportion of winged forms secured from *Myzus ribis* by growing the aphid on currant cuttings placed in MgSO_4 solution. She

found that a larger proportion of winged forms appeared on cuttings placed in tap-water (no analysis was given) than on those placed in a solution of MgSO_4 . Because of the high percentage of winged forms appearing among the tap-water controls, Miss Haviland doubted the effectiveness of magnesium sulphate.

No marked increase of winged forms was secured by Mason ('22) when he reared the green rose aphid (*Macrosiphum davisii*) on rose cuttings placed in sand moistened with 1 per cent and 5 per cent MgSO_4 solutions, even though they were carried through three generations, thus permitting a cumulative effect of the salt. *Myzus persicae* grown on rose cuttings gave similar results. During his experiments, Mason changed the aphids to fresh rose cuttings every three days. Wadley ('23) found that the watering of wheat seedlings grown in soil with solutions of various salts failed to increase the proportion of winged forms. He also grew a small number of aphids on wheat seedlings grown in sand moistened with solutions of Mg, Zn, Cu, and Pb salts, and also on wheat cuttings placed in salt solutions. By no method was he able to secure results that were at all comparable with those of Shinji, and it is evident that his results are far from indicating that certain salts taken in with the food of aphids are more effective than others in inducing wing production.

Unfortunately, in much of the work with salt solutions insufficient care has been taken to use solutions that are not too highly toxic. If the solution is sufficiently strong to kill or almost kill the plant, it becomes almost impossible to distinguish between the supposed wing-developing effects of the salt as a component of insect food and the effect of feeding on plants that are either dead or suffering from a serious disturbance of their metabolism as a result of the toxicity of the salt solution.

As is well known, the metallic salts are much more effective in their action on protoplasm than salts of Na and K, or even the salts of Ca, Sr, Ba, and Mg (True and Gies, '03; Hawkins, '13). Thus while a m/20 solution of a Na or K salt is not

likely to kill green plants, a m/20 or a m/50 solution of Mg or a m/1000 solution of Zn, Cu, Pb, Sb, or Ag salts is almost certain to be a killing concentration. Clarke used saturated solutions of MgCl_2 , MgSO_4 , K_3PO_4 , and NaH_2PO_4 . It is very unlikely that the potassium salt was of a killing concentration, the sodium salt may have been, but the magnesium salts could hardly have been otherwise. A m/10 concentration was used by Wadley, except when the limits of solubility prevented, in which case he used saturated solutions.

For the most part, it is not clear just what concentrations were used by Shinji. He recognized that the more concentrated solutions of certain salts were toxic to the rose cuttings that he used, and states that "only a few drops of the normal solutions of these salts were added to the 5 cc. of distilled water, in most of my experiments." A m/40 solution of MgSO_4 is referred to as being an 'ordinary strength' of wing-producing substance.

Granting, however, that all of Shinji's 'wing-developing' substances may have caused the death of the rose cuttings, and that, for the most part, his 'non-wing-developing' substances did not kill the cuttings, we have not by any means weakened Shinji's general thesis that certain salts taken in with the food of aphids induce wing production, while other salts have an opposite effect, for Shinji clearly states (pp. 98 and 101) that, although aphids grown on cuttings in Ca, Sr, Hg, Pb, and Cu solutions were greatly weakened and frequently lost because of the toxic effect of the salt solutions, less than 1 per cent of the aphids grown on the non-wing-developing substances (Ca and Sr) were winged, while approximately 90 per cent of the aphids from cuttings in the wing-developing substances (Hg, Pb, and Cu) were winged.

Experiments with salts

I have been unable to confirm the results of Clarke, Neils, and Shinji. Over 4000 aphids were raised during a set of preliminary experiments in an endeavor to increase the percentage of wing production by modifying the nature of the

salt solution. MgSO_4 , ZnSO_4 , AlCl_3 , MnCl_2 , Ni acetate, and CuSO_4 , as well as KNO_3 and KH_2PO_4 , were used in a wide variety of concentrations and combinations. The plants for the most part were used immediately after germination, but several hundred aphids were also raised on plants which had been growing in their respective solutions for the usual period of four days. It was found that when aphids were reared on plants grown in solutions of the 'wing-developing' salts winged forms did not appear in any larger proportions than when they were reared on plants growing in the usual nutrient salt solution.

When the temperature experiments were completed, tests were made to compare the effects of a 'non-wing-developing' substance, KNO_3 , and a 'wing-developing' substance, MgSO_4 . Plants were placed in jars containing the respective solutions for four days before the wingless aphids which were used as parents were placed on them, and new plants were provided only to replace those that had died. Overcrowding was permitted in all cases and to an approximately equal extent. The parents themselves were derived from overcrowded sources. The results given in table 4 are all approximations and represent the condition at the end of two weeks; that is, during the second generation. Winged aphids occurred on plants grown in KNO_3 solution fully as frequently as on plants grown in MgSO_4 . This is especially true of aphids reared on plants growing in the stronger concentrations of KNO_3 .

When parents derived from sources that gave assurance of their being well fed were reared on plants growing in these same concentrations of KNO_3 and MgSO_4 , only four winged forms appeared in the first generation. Two of these were on plants in KNO_3 and two on plants in MgSO_4 solutions.

These results show that in so far as the oat aphid is concerned, winged forms appear on plants growing in KNO_3 as well as on plants growing in MgSO_4 solutions if the parents are derived from overcrowded sources and the offspring are raised under overcrowded conditions, and that when parents are derived from well-fed sources fewer winged offspring

appear, but that they are just as likely to occur on plants grown in KNO_3 solutions as on plants in MgSO_4 solutions. These results thus indicate that any influence that salts may have as direct agents of wing production is negligible as compared with starvation.

Approximately 1000 aphids were raised with more than usual care through three or four generations on plants growing in various salt solutions at 24° . The plants as usual were kept in their respective salt solutions for four days before being used. A single aphid or two were placed on each plant within twenty-four hours after the aphids were born. When the aphids became adult they were removed and measured,

TABLE 4
Comparison of effects of KNO_3 and MgSO_4

SALT	CONCENTRATION					
	m/10	m/50	m/100	m/500	m/2000	m/2500
KNO_3	About 80 per cent winged Plants died	About 60 per cent winged	About 30 per cent winged		About 25 per cent winged	
MgSO_4	Plants died	About 40 per cent winged Plants died	About 50 per cent winged Plants died	About 40 per cent winged		About 20 per cent winged

and their colors were described. After the first generation the aphids used were always obtained from plants growing in the same kind of solution, thus permitting a cumulative effect of the salts through the several generations. The progenitors of all these aphids had lived at 24°C . for several months.

Solutions A and B were of the same composition as those described in the section on methods and apparatus. Solution C was a m/10 solution of Mg_2SO_4 . The following combinations were used: 1) Distilled water. 2) Various concentrations of equal quantities of solutions A and B. The following numbers of milliliters of solutions A and B were used with

enough water to make up a liter of solution: $2\frac{1}{2}$, 5, 10, 20, 40, 75, 100, 125, 150, 200, and 400. 3) Unbalanced solutions of A and B as follows: a) 300 parts sol. A, 100 parts sol. B, 600 parts H_2O ; b) 300 parts sol. B to 700 parts H_2O . 4) Unbalanced solutions containing $MgSO_4$ as follows: a) 10 cc. sol. A, 10 cc. sol. B, 450 cc. sol. C, 530 cc. H_2O ; b) 15 cc. sol. A, 15 cc. sol. B, 450 cc. sol. C, 520 cc. H_2O ; c) 25 cc. sol. A, 25 cc. sol. B, 450 cc. sol. C, 500 cc. H_2O ; d) 200 cc. sol. A, 80 cc. sol. B, 160 cc. sol. C, 560 cc. H_2O ; e) 500 cc. sol. A, 25 cc. sol. B, 475 cc. H_2O . 5) The following concentrations of KNO_3 : m/5, m/10, m/50, m/100, m/500, m/1000, and m/5000. These concentrations or proportions for solutions of groups 2, 4, and 5 ranged from killing concentrations to very dilute or non-toxic concentrations. The solutions of group 2 were all started at a pH of approximately 6.0, except at the higher concentrations where such a pH caused precipitation to occur.

No winged forms appeared on plants in any of the solutions of groups 1 and 4. One winged form appeared on plants in solution b of group 3 and one on the dead plants in a m/5 solution of KNO_3 . Five per cent of the aphids raised on plants grown in solutions of group 2 were winged, they being distributed rather evenly throughout the range of concentrations used, but with some indications of occurring in highest proportion where 40 cc. of solutions A and B were used.

The differences of color and body length which were observed during the course of this experiment occurred too irregularly to warrant comment.

Thus it was found once again that aphids grown on plants in $MgSO_4$ solutions were no more likely to produce winged forms than if the plants were grown in physiologically balanced solutions or in solutions of KNO_3 .

Discussion of salt experiments

The question of whether or not certain salts induce wing production while other salts act as inhibitors is still unanswered. It must be remembered that in my experiments a limiting factor may enter which prevents a sufficient altera-

tion of the salt content of the food of the aphids. It may be that the plant is unable to absorb a sufficient quantity of the 'wing-developing' type of salt to affect the production of wings without killing the plant. This factor might not be involved when rose aphids are raised on cuttings. However, the appearance of the winged form in large proportion during my experiments when a 'non-wing-developing' substance, KNO_3 , was used suggests that Shinji, Clarke, and Neils may have taken insufficient account of parentage, starvation, and temperature conditions.

CHARACTERS CORRELATED WITH WING PRODUCTION

The grain aphid in both the winged and wingless form molts four times before the adult stage is reached. The various instars may be identified by the number of antennal segments and the appearance of the cauda. At birth the antennae have four segments; in the second instar they have five segments; in the third instar, six segments, and in the fourth instar, six segments, but the aphid has no cauda. The adult has a well-developed cauda and antennae with six segments.

Ocelli and sensoria

During the first instar the winged and wingless forms are not distinguishable. Thoracic protuberances, the first sign of developing wings, make their appearance in the second, third, or fourth instars. In the fourth instar those aphids which have wing pads also show well-marked, but not fully developed ocelli. In the adult winged aphid the ocelli are well developed, and approximately twenty-eight sensoria appear on each of the antennae. Sensoria have never been seen during these experiments in wingless aphids. Rudimentary ocelli are occasionally present in adult aphids having thoracic protuberances, but no wings or stubs of wings.

Cuticle

The cuticle of adult winged aphids is smooth and unmarked except for a few spines posteriorly. In adult wingless

aphids, however, the cuticle is strongly marked and resembles the cuticle of the nymphs of both types. These markings have the appearance of being impressions of the underlying hypodermal cells. The cell-like outlines are due to a broken, rather than a solid, line, the breaks causing the outline to have a beaded appearance. When viewed from the side, it can be seen that these beads are more or less strongly developed protuberances that frequently project enough to be properly called spines or bristles. Wingless aphids show all grades of markings from a condition wherein beads and spines are almost wholly absent to those cases where both beads and spines are strongly developed. The first condition, however, occurs very infrequently.

Intermediates

Intermediates are aphids that have started to develop wings, but fail to complete such development. The partially developed wings and wing muscles usually undergo some degeneration and wing pads or stumps of wings are present in various degrees of formation. Rudimentary ocelli are almost always present, even though the process of wing development has gone no further than to change slightly the shape of the thoracic region. The sensoria are usually weak and irregular or absent. In one case fifteen sensoria were counted on one antenna, although none were present on the other.

Klodnitski ('12) pointed out that the development of the wing anlagen may be checked by external influences and especially by placing the aphid on a fresh plant. The intermediates of Turner and Baker ('15) also appeared on young plants. Ewing ('16) noted the occurrence of intermediates, but attributed to them a paedogenetic status.

During the course of my experiments intermediates appeared frequently as a result of putting young aphids on freshly germinated seedlings, and I believe that the failure of wings to complete their development may be attributed primarily to this environmental change. It has already been

suggested that winged forms appear much less readily on freshly germinated plants than on older plants, because of differences in the proportion of water to carbohydrates, etc., as they occur in the food of aphids. If this view is correct, it would seem that intermediates are the result of increased dilution of the body fluids, presumably (in view of facts presented in other sections) the haemolymph.

Size

Taxonomic descriptions of aphids usually attribute a smaller size to the winged adult than to the wingless adult. Shinji ('18) and Wadley ('23) also indicate that their winged adults were smaller than their wingless adults. During the course of these experiments, the body length of more than 1000 aphids was measured, mostly of aphids grown at 24°C. The wingless aphids at this temperature were found to range from 0.96 mm. to 2.14 mm. in length; the adult winged aphid ranged from 1.08 mm. to 2.2 mm. This range is very much wider than has been previously reported by any one other than Ewing ('16).

Two significant facts were learned from these measurements: 1) Although the body lengths of both winged and wingless grain aphids cover a wide range, the body length of the adult winged aphid was consistently slightly longer (about 10 per cent) than the average length of its sisters, and usually longer than that of any of its sisters, if they were raised under identical conditions. 2) Aphids raised on freshly germinated seedlings, one aphid to a plant, were approximately 25 per cent longer than those grown on plants that were four days old, but under conditions otherwise similar (Ewing, '16, made similar observations), and from 70 per cent to 100 per cent longer than aphids grown on old plants under overcrowded conditions.

As previously stated (discussion of starvation experiments), these relationships are believed to be concerned with the proportion of water to carbohydrates, etc., as they occur in the food of aphids.

Coloration

Coloration in the grain aphid is almost entirely due to the presence of two pigments which are present in certain of the globules in the haemolymph. These globules are so located as to give to the dorsal aspect of wingless aphids two bands of color. A green band extends from approximately the anterior margin of the thorax to a point slightly anterior to the insertion of the cornicles. A brown band, which is the narrower of the two bands, extends across the abdomen in the region of the cornicles. The coloration of these aphids varies with temperature, with the form of the individual (winged or wingless), and with their age.

The two colors as seen in the wingless form are oppositely affected by temperature changes. The brown coloration becomes more prominent as the temperature is lowered, but the green coloration becomes deeper and more prominent as the temperature is raised. At approximately 16°C. and below, the green coloration of the oat aphid is subordinate to the brown color and at times appears to be wholly absent. On the other hand, aphids that have lived at 29°C. for a few generations are a deep dark green and the brown globules may be wholly or almost wholly absent. In general, also, it is noticeable that aphids of the first generation at low temperatures are likely to be a stronger brown than aphids of later generations at these temperatures.

It is only at temperatures of 16° and below, so far as I have observed, that the brown globules frequently have among them a small number of globules that are green instead of brown, but which are identical with the brown globules in all other respects. It has also been observed that when aphids are subjected to severe starvation by overcrowding, a few brilliant red globules, usually of very small size, may be interspersed with the green globules. Under no other conditions have globules containing pigments of different colors been observed in the same color band.

As development of the winged form proceeds, a slight diminution in the extent of the brown band becomes noticeable up to the last molt. After this molt, that is, with the appearance of the fully developed wings, the globules containing the brown pigment have either entirely or almost entirely disappeared. In well-fed wingless aphids there is no perceptible decrease in the number of brown globules during development. The significance of the disappearance of the brown globules in the winged form and further consideration of the characters correlated with wing production will be deferred to later sections.

THE HAEMOLYMPH AND WING PRODUCTION

Introductory

An examination into the nature of aphid coloration was instituted because of the relationships that exist between the coloration of aphids and wing production. This examination was soon extended to include most of the elements of the haemolymph, which in these aphids makes up about one-quarter of the total bulk of the insect.

Four distinct kinds of globular structures may be observed in the haemolymph, two of which are pigmented, the other two being colorless. No attempt will be made at this time to discuss the morphology and derivation of these structures, and the descriptive terms that are used herewith are employed without prejudice to their morphological significance.

As has already been pointed out, the two types of pigmented globules (green and brown) are restricted to definite regions. The brown globules occur in the region of the cornicles. The green globules are anterior to the brown globules. In size the brown globules range from 2μ to 8μ in diameter, most of them being about 4μ . The green globules are smaller and have a diameter of 1.5 to 3μ .

Colorless globules occur in each of the two color bands. Treatment with sudan III and with osmic acid indicated that they consist primarily of fatty or lipoidal substances. Inasmuch as the colorless globules of the two color bands do not

possess identical characteristics, it is necessary to differentiate between them. The colorless globules of the brown color band increase in size during the development of the aphid, but in the adult wingless form they have an average diameter of about 40 μ . They are larger than those of the green region and will be referred to as the large fat-globules. The colorless globules of the green band will be referred to as the small fat-globules.

A definite and peculiar relationship exists between the colored globules and the fat-globules, especially in the brown color band. The large fat-globules are always completely surrounded, or almost so, by from one to three or four layers of closely packed brown globules. A somewhat similar but less definite relationship exists between the green globules and the small fat-globules. This study, however, has been concerned primarily with the globules of the brown color band, and no detailed description of the conditions in the green color band will be attempted.

Relation of the fat-solidification temperatures of the large fat-globules to habitat temperatures

Wingless aphids of a line that had been living at a temperature of 24° for several generations were placed in a small stoppered glass vial and submerged in water having a temperature of 8° for a period of one hour. The aphids were then removed, crushed under a cover-slip, and examined with a microscope. The large fat-globules were seen to coalesce readily and then, after a few seconds to a minute or so, it was observed that the fat-globules solidified. The fat-globules, in other words, had remained liquid notwithstanding the exposure to a temperature of 8°, but had solidified as a result of changes caused by the crushing of the aphid.

When, however, 24° wingless aphids were exposed to a temperature of 7° for one hour many of the large fat-globules did not coalesce when the aphid was crushed, and upon being subjected to increased pressure, they cracked. That is, many

of the large fat-globules had solidified as a result of the exposure to 7°. Exposure to 6° caused all of the large fat-globules to solidify.

Aphids that had been reared for several generations at 28°, 18°, 16°, and 12° were similarly treated. As may be seen in table 5, the fat-solidification temperature was different for each temperature at which the aphids had been living.

TABLE 5

Relation of fat-solidification temperatures to habitat temperatures. Ap, apterous; W, winged; L, globules all liquid; S, globules all solid; B, both liquid and solid globules are present; B^s, globules almost all solid

EXPOSURE TEMPERATURES	HABITAT TEMPERATURES						
	28°	24°		18°	16°		12°
	Ap	Ap	W	Ap	Ap	W	Ap
<i>Degrees</i>							
10	L						
9	B	L					
8	S	L	L				
7	S	B	L				
6		S	B				
5		S	B	L			
4			B ^s	L	L		
3			S	B	L	L	
2			S	S	L	L	
1				S	B	L	
0					S	B	L
-1					S	B	L
-2						B ^s	B
-3						S	S

By the term habitat temperature is meant the temperature at which an aphid and its progenitors have lived continuously and without variation for several generations. The term solidification temperature refers to the temperature at which the large fat-globules solidify. By exposure temperature is meant the temperature to which aphids were subjected for one hour to determine their fat-solidification temperatures.

Globules which have become solid remain so for an extended period of time. Aphids having a habitat temperature of 24°C . were placed at 0°C . for one hour, to solidify the fat-globules, and were then returned to a temperature of 24°C . These aphids lived and reproduced normally. On the fourth day, after being returned to 24°C ., all of the fat-globules were still solid. On the fifth day some liquid globules, possibly of recent origin, were present. On the seventh day some of the fat-globules were found to be still solid.

It is clear that the fat-solidification temperature is not the same as the melting-point of the fat. Aphids from the 24° chambers were placed at a temperature of 6° to solidify the fat-globules and were then subjected while in the glass vial to warmer temperatures. It was found that a temperature of approximately 65° was necessary to melt the fat-globules within a period of one hour. That is, globules which had solidified at a temperature of 6° , liquefied at a temperature of about 65°C . An explanation of this striking difference is suggested in a later section of this paper.

Whatever may be its physicochemical nature, the solidification of the fat-globules occurs with a remarkable degree of precision. Of twenty-one wingless aphids having a habitat temperature of 24°C . that were exposed to a temperature of 8°C ., all of the globules in twenty of the aphids were liquid. In the one exception only two or three of the several hundred globules present were solid. When an exposure temperature of 7°C . was used, the globules in twenty-two aphids out of twenty-seven were all or almost all solid; in three aphids, only a small number were solid, and in the other two aphids none of the fat-globules had solidified. When an exposure temperature of 6°C . was used, all the fat-globules in nineteen aphids out of twenty solidified, while in the one exception only a few of the globules remained liquid.

Relation of fat-solidification temperatures to the form of the aphid (winged or wingless)

The winged form, as may be seen from the data in table 5, differs from the wingless form with regard to the solidification of the fat-globules in two ways, namely, 1) the solidification temperatures of the fat-globules in winged aphids are from 1° to 3° lower than those of wingless aphids. 2) The globules in a single aphid of the winged form are more variable than those of wingless aphids. Thus in winged aphids (the nymphal form was mostly used in these tests) having a habitat temperature of 24°C. none of the fat-globules solidified at an exposure temperature of 7°C. At 6°, 5°, and 4°, some of the globules solidified, while others remained liquid. At 3° all were solidified. In winged aphids having a habitat temperature of 16°, none of the fat-globules solidified at an exposure temperature of 1°. Some of the globules solidified at 0°, -1°, and -2°, while other globules remained liquid. At -3° and below, all the fat-globules solidified. These solidification temperatures are, as indicated in the table, about 2° below those of the globules in wingless aphids raised at the same temperatures. It is evident, then, that some sort of relationship exists between wing production and the solidification of the fat-globules.

Time required for arriving at a condition of thermal equilibrium

For each habitat temperature, as shown above, there is a definite and different fat-solidification temperature. Some sort of adjustment must therefore occur when aphids are changed from one temperature to another. It may be said that when aphids have been at a given temperature for a sufficient length of time to complete this adjustment, they are, in so far as the solidification of fat-globules is concerned, in a state of equilibrium with their thermal environment.

When aphids are changed from a habitat temperature with which they are in a state of equilibrium to a new habitat

temperature, the time required for arriving at a condition of equilibrium with the new thermal environment depends upon whether or not overcrowding is permitted and upon whether the tests are made on the original aphids or upon their offspring. This is shown by the following experiments.

Aphids from a line that had been reared at a temperature of 24°C. for several generations were transferred to a temperature of 16°C. It was found that when only one or two aphids were allowed to live on a single plant, the original aphids gradually approached a condition of thermal equilib-

TABLE 6

Change of fat-solidification temperatures in wingless aphids transferred from 24° to 16° with no overcrowding. B¹, globules almost all liquid.

Other symbols as in table 5

EXPOSURE- TEMPERA- TURES	DAYS AT 16°C.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>Degrees</i>														
8	L	L	L	L										
7	B	B	B	B ^s	L	L	L							
6	S	B ^s	B	B	B ¹	L	L	L	L					
5	S	S	B	B	B	B	B ¹	L	L	L	L	L		
4		S	B	B	B	B	B	B	B ¹	B	B	B	L	L
3			B ^s	B	B	B	B	B	B	B	B	B	B	B
2			S	S	B	B	B	B	B	B	B	B	B	B
1			S	S	S	B	B	B	B	B	B	B	B	B
0					S	S	S	S	S	S	S	S	S	S

rium with the new environment, but did not reach it even after fourteen days had elapsed. The change as followed day by day is set forth in table 6. On account of the aging of the aphid, it was not considered advisable to attempt to secure data beyond the fourteenth day.

Aphids transferred from 16° to 24°C. under similar conditions underwent a similar change, but in the opposite direction. The results are given in table 7.

To determine the effects of overcrowding on the time necessary for aphids to arrive at a state of equilibrium with their

new thermal environment, twenty to thirty aphids were allowed to grow on a single plant. The aphids were derived from the 24° chamber and were transferred to 16°. Only the original aphids were tested. A comparison of the data in table 8 with that of table 6 shows that aphids living under overcrowded conditions proceeded approximately as far during four days as aphids not overcrowded, but otherwise, similarly treated, proceeded in eight days. Furthermore, aphids raised under overcrowded conditions reached a state of equilibrium on the ninth day, while aphids which were not overcrowded, but subjected to otherwise similar conditions, did

TABLE 7

Change of fat solidification temperatures in wingless aphids transferred from 16° to 24° with no overcrowding. Symbols as in tables 5 and 6

EXPOSURE TEMPERATURES	DAYS AT 24°C.								
	1	2	3	4	5	6	7	8	9
<i>Degrees</i>									
8						L	L	L	L
7		L	L	L	L	L	B	B	B
6		L	L	B	B	B	B	B	B
5	L	B	B	B	B	B	B	B	B
4	L	B	B ^s	B ^s	B	B	B	S	S
3	B ^l	B ^s	S	S	S	S	S		
2	B	S							
1	S								

not reach an equilibrium condition even on the fourteenth day, and apparently would not arrive at such a condition during the lifetime of the aphids.

Inasmuch as overcrowding served to hasten the change in the fat-solidification temperatures of aphids which were transferred from 24° to 16°, it might appear (Issakowitsch, '05, for the effect of constant temperatures on daphnids) that the effect of temperature is primarily due to changes in the food supply rather than directly on the aphids. Observations of two different kinds indicate that this cannot be true in so far as the fat-solidification temperatures are concerned. Although the nature of the food supply at a given tempera-

ture must vary between rather wide limits, the fat-solidification temperatures of aphids raised at a given habitat temperature differ from one another much less than do the solidification temperatures of aphids grown at different habitat temperatures. Aphids grown at a temperature of 24°C. cannot be so affected by a change in their food supply as to depress their fat-solidification temperatures nearly so low as the highest solidification temperature obtainable in aphids grown at 16°C.

Furthermore, aphids were transferred from a temperature of 24° to 16°C. as in previous experiments, but provided

TABLE 8

Change of fat-solidification temperatures in wingless aphids transferred from 24° to 16°; overcrowded. Symbols as in tables 5 and 6

EXPOSURE TEMPERATURES	DAYS AT 16°C.								
	1	2	3	4	5	6	7	8	9
<i>Degrees</i>									
8	L	L							
7	B	B ^l							
6	B	B	L	L	L				
5	B	B	L	L	L	L			
4	S	S	B	B ^l	B	L	L	L	
3	S	S	B	B	B	B	L	L	L
2			B	B	B	B	B	B ^l	L
1			S	B	B	B	B	B	B
0			S	S	S	S	S	S	S

daily with fresh plants that had been germinated and grown at 24°C., instead of being required to live on the same plants throughout their lives. After a period of seven days had elapsed, the fat-solidification temperatures of these aphids was determined. At an exposure temperature of 7° and 6°C. all the fat-globules remained liquid. At 5°C. in some aphids all the fat-globules were liquid, while in other aphids some of the fat-globules were solid. At 4°, 3°, 2°, and 1°C. both solid and liquid globules were present in all aphids. At 0°C. all the large fat-globules were solid. There was thus no appreciable difference between aphids that were provided with

fresh plants daily and those (table 6) which had remained on the same plant throughout the period.

The above observations lead me to believe that the effects of temperature with respect to changes in the fat-solidification temperature are primarily on the aphids themselves, and not on the plant. The fact that a condition of thermal equilibrium was approached more rapidly when aphids were reared under overcrowded conditions (table 8) than when they were reared without overcrowding (table 6) merely indicates that severe starvation affects the fat-solidification temperatures of aphids, while the minor changes of food supply, such as may accompany changes of temperature, have little or no effect on the fat-solidification temperatures.

TABLE 9

Change in the fat-solidification temperatures of the offspring of aphids transferred from 16° to 24°; no overcrowding. Symbols as in tables 5 and 6

EXPOSURE TEMPERATURES	DAYS AT 24°C.				
	2	3	4	5	6
<i>Degrees</i>					
8	L	L	L	L	L
7	B ¹	B	B	B	B
6	B	B	B	B ^s	B
5	B ^s	S	S	S	S
4	S				

The offspring of aphids transferred from 16° to 24° and raised under conditions which prevented overcrowding were tested for their fat-solidification temperatures. The results are given in table 9. When aphids that were themselves transferred from 16° to 24° were similarly tested (table 7), a condition of thermal equilibrium was not yet attained on the ninth day and probably would not have been reached before death from old age. For the offspring of aphids so treated the adjusting process was completed on the sixth day after the parents were transferred to 24°. On the second day (the first day on which it was possible to make a test) the offspring which were then in the first and second instars had progressed

further with the adjustment than the original aphids had progressed in seven days. Aphids born at the new temperature thus arrived at a condition of thermal equilibrium sooner than did their parents.

An interpretation of the causes of fat solidification and the relation of these data to wing production is discussed in later sections.

Chemistry of the fat-globules

Since Büsgen ('91), with the assistance of the German chemist, Professor Knorr, concluded that the fat-globules of aphids (*Aphis rosae* and several other species were used) are a waxy substance, it has been customary to refer indiscriminately to the cornicular discharges of aphids as being a waxy material. Büsgen states specifically, however, that the pigmented mass which accompanies the fat-globules was not included in his observations. There can be no doubt that the waxy substance to which he referred is the lipid material of the fat-globules and presumably of the large variety.

I have been able to confirm all the tests used by Büsgen and Knorr, namely, insolubility in water and cold alcohol and solubility in hot ether and hot alcohol (with crystallization on cooling), on the basis of which they concluded that the lipid is a wax or a wax-like substance. Obviously, such tests are insufficient for a conclusive determination. Inasmuch as the lipoids of an aphid, *Psylla alni*, and of various coccids have been subjected to a more rigid chemical analysis (Lewkowitsch, '22), however, and it has been found that they are waxes, it seems probable that the determination of Büsgen and Knorr will stand. The waxes are noted for their remarkable powers of emulsification, and this property may be of special significance in many of the reactions that occur in the haemolymph.

The small fat-globules which are associated with the green globules are not of the same composition as the large fat-globules, and they may not be waxes. Live aphids under a cover-slip were treated with a drop or two of glacial acetic

acid and then crushed. The large fat-globules formed large, colorless to grayish, spheroidal clusters of needle-shaped crystals. The small fat-globules, on the other hand, usually coalesced to form much larger globules and then with considerable rapidity completely dissolved, or at most left only a small residue.

The large fat-globules coalesced readily in aphids that were crushed but otherwise untreated. The small fat-globules underwent coalescence much less frequently when so treated.

Conclusions regarding the large fat-globules

From the foregoing data it is believed the following conclusions may be drawn: 1) The fat-solidification temperature is different for every temperature at which the aphid lives. 2) As shown by their fat-solidification temperatures, aphids arrive at an equilibrium with their thermal environment if they are kept at a constant temperature for a sufficient length of time. 3) The time required for an aphid to arrive at a condition of thermal equilibrium varies from a few days to more than two weeks, and equilibrium apparently is not reached during the life of the original aphid. The time required is dependent on, *a*) the amount of overcrowding and, *b*) whether the original aphids or their offspring are tested. It is probable, also, that the time required for adjustment varies with the specific temperatures involved. 4) With respect to differences in the fat-solidification temperatures of aphids grown at different habitat temperatures, the effect of the temperature is primarily on the aphids themselves, and not on the plant. 5) The fat-solidification temperatures of winged aphids are from one to four or more degrees lower than those of wingless aphids having the same habitat temperature. 6) The solidification temperature of a given fat-globule undergoes a gradual change with changes in the habitat temperature. 7) The solidification temperatures of some fat-globules change more rapidly than do those of other fat-globules in the same aphid. 8) There is probably some

fundamental relation between wing production and the solidification temperatures of the fat-globules. 9) The lipoid in the large fat-globules is probably a wax, although the evidence is inconclusive.

Nature of the brown globules

A very striking peculiarity of the brown globules is the ease with which they break when subjected to disturbing conditions. When live aphids were crushed under a cover-glass and the haemolymph was pressed out of the body cavity most of the brown globules were disrupted during the process. Some, however, persisted for as long as an hour or more if left undisturbed. Chemical and thermal disturbances resulted similarly in their disruption.

Although several hundred brown globules were subjected to close individual scrutiny, they have never been observed to coalesce. In each and every case the globules have finally broken, the break occurring instantaneously, and the globules were observed to be completely miscible with the surrounding media. Coincident with disruption the globules disappeared without leaving any trace or visible residue.

It is apparent that the brown globules must possess some sort of confining membrane. This membrane might conceivably be explained as being a very delicate precipitation membrane, the maintenance of which is dependent upon the preservation of equilibrium conditions between the substance in the membrane with both the surrounding haemolymph and with the substances that are enclosed by the membrane. When this equilibrium is destroyed as by the dilution of the haemolymph, by the addition of chemicals, or by thermal changes, it might be considered that the bounding membrane breaks and the globule mixes with the haemolymph.

It is also conceivable, however, that these globules represent dead cells which have undergone extreme autolysis. I do not believe that this conception represents the true nature of these structures, but an expression of opinion is probably premature, for even a discussion of this possibility must await further investigation.

In any case, it cannot be too strongly stated that the brown globules of these aphids have no semblance of cellular structure, but rather that they appear to be globules of a solution which for physicochemical reasons have not mixed with the haemolymph. When they disappear, they do so as completely and as suddenly as does a droplet floating on a surface of boiling salt solution.

Occurrence of the brown globules in winged and wingless aphids

As has been noted in a previous section, the brown globules are wholly or almost wholly absent in the adult winged aphid, although they are present in large quantity in the nymphs of both forms and in the adult wingless aphid. There are two possible ways of accounting for the disappearance of the brown globules during the final molt of winged aphids. The brown globules may be expelled through the cornicles or their disappearance may be due to a disruption of the confining membrane. My observations seem to preclude any other possibility. A small amount of material is discharged through the cornicles in all forms when the aphid is disturbed, but there is no evidence of any unusual discharge at the time of the last molt in winged aphids.

During the early stages of wing development the haemolymph in the region of the wing buds frequently has a purplish tinge. The addition of a few drops of a dilute alkaline solution caused this color to become a more brilliant red. In like manner, the brown globules themselves also became a brilliant red when treated with dilute alkaline solutions. The reaction of the haemolymph in the region of the developing wing buds to dilute alkalies, in other words, is the same as is the reaction of the pigment in the brown globules to dilute alkalies. There can be little doubt but that the brown globules disappear during wing production as a result of their disruption and that the contents of the globules are dissolved in the haemolymph.

When an adult winged aphid was immersed in concentrated NaOH, however, the red color that developed was much less intense than that which developed when adult wingless aphids were similarly treated. Thus not only have the brown globules disappeared, but the pigment itself appears to have diminished in quantity. It is apparent that the pigment has been converted into some other material.

The nature of the brown globules thus takes on a new importance. Krukenberg ('86) suggested that, because of the glucosidal nature which he attributed to the brown pigment of aphids and because of the large amount of pigmented material present in the female, the coloring matter of aphids probably functioned as a reserve food. Newbigin ('98) criticised Krukenberg's theory on the grounds that chitin itself is a derivative of carbohydrates and is present in large quantity, but that no one has suggested that it is a reserve food.

Can it be that Krukenberg's theory expressed a half truth? May not the development of wings in aphids be dependent upon the amount or concentration of materials in the haemolymph as determined by the breaking of the brown globules? Obviously, it is not possible to give a conclusive answer to this question. Numerous facts indicate that such may be the case. Nevertheless, no matter how convincing these facts may appear to be, no judgment should be rendered until more evidence has been obtained and especially from other species of aphids.

The chemical nature of the pigment

Widely divergent conclusions regarding the chemical constitution of the brown pigment have been reached. Sorby ('71) appreciated the similarities that exist between the cochineal of commerce and the brown pigment of aphids, but he was inclined to believe that the brown coloration of aphids is due to the presence of a haemoglobin. Krukenberg ('86), however, interpreted Sorby's data as indicating that Sorby had a mixture of carminic acid (the active principle of cochineal) and a lipochrome. Buckton ('76) pointed out that

there is no essential difference between the green and brown pigments, and concluded that the coloring matter of aphids partakes more of the nature of chlorophyll than of derivatives of carminic acid. Two papers on the pigments of aphids were published by Bêlousov ('06-'07) which I have not as yet been able to obtain. His work has never been quoted and I am wholly unaware of its nature. Uichanco ('24), who discussed the pigments of the 'symbiotic' organs of aphids, apparently follows Buckton in believing the pigment to be chlorophylloid. In 1924, Palmer and Knight concluded, with some uncertainty, however, that pigmentation in aphids is due to the presence of anthocyanins.

The observation by Mayer ('92), that the coloring matter of the cochineal insect, *Coccus cacti*, occurs in drops around the periphery of the fat-cells, lends color to Krukenberg's contention that the pigments of aphids are of the same chemical nature as is cochineal. Krukenberg ('86) states that the pigments of the 'carminic acid series' are limited in the animal kingdom to coccids and aphids.

Perkin and Everest ('18) accept Dimroth's view that the active principle of cochineal, carminic acid, is a derivative of anthraquinone. Laccaic acid and kermesic acid, which are obtained from two other species of coccids, have also been identified as being derivatives of anthraquinone.

I have found that when an ether extract of the brown pigment of chrysanthemum aphids, *Macrosiphum sanborni*, was shaken with distilled water, the pigment remained almost entirely in the ether layer. When, however, the water was made slightly alkaline with a drop of ammonia, the brown pigment readily washed out of the ether. A drop or two of dilute acid added to the alkaline solution changed its color from a bright red to a pale yellow. The addition of concentrated glacial acetic acid caused the solution again to become red. A bright red alkaline solution became almost colorless on standing overnight.

The pigment of the grain aphid was also extracted with ether and washed with distilled water. A drop of ammonia

added to the water solution caused its color to change from a greenish yellow to an orange yellow. This color deepened when the solution was allowed to stand overnight and deepened more rapidly when a stream of oxygen was passed through it. After standing another day or so, however, the solution became quite colorless.

When the washing and shaking referred to above was carried on too vigorously, an extremely thick and persistent emulsion of water and ether was formed which presumably involved the fat (wax?) that was present.

All attempts at crystallization of the pigment, except by microchemical means to be described shortly, were unsuccessful.

It is evident that the pigment is a compound possessing a considerable degree of instability. In its various states it may be soluble in ether, alcohol, dilute alkaline solutions, and strong acid solutions, but be comparatively insoluble in very dilute acid solutions. The pigment apparently is an organic acid. It may undergo oxidation readily (and reduction?) (also see Sorby, '71). It has been impossible up to the present to cause the pigment to crystallize except by microchemical methods from concentrated nitric acid. In all these respects the pigment behaves like the pigments of the carminic-acid group.

The Teichmann test for haemoglobin gave negative results repeatedly.

It would seem as though the breaking up of the brown globules, resulting as it does in the discharge of their contents into the haemolymph and the liberation of the enclosed fat-globules, thus projecting into the haemolymph comparatively large quantities of new materials, might well influence the development of wings, sensoria, and ocelli.

Relation of the brown globules to the large fat-globules

The following observations have caused me to believe that the brown pigment and probably other substances from the brown globules are soluble in the large fat-globules. Wing-

less aphids were crushed under a cover-glass and, after waiting a few moments, a drop or two of concentrated nitric acid were added. The large fat-globules gradually became a very intense brilliant red, and after a short time the color gradually became brownish to colorless. Both in the fat-globules and in the surrounding medium small brown sheaf-like clusters of crystals appeared which are believed to represent the brown pigment. When aphids were treated with nitric acid immediately after crushing, that is, without sufficient time being allowed for the contents of the disrupted globules to dissolve in the fat-globules and possibly for other changes to take place, the color that appeared in the fat-globules as a result of the application of nitric acid was very weak or wholly absent. Moreover, when aphids were killed by heat or by ether fumes, thus causing the brown globules to break and permitting their contents to dissolve in the fat-globules while still within the shell of the aphid, the color of the fat-globules which resulted from the application of nitric acid was very intense.

By previously subjecting the aphid to low temperatures to solidify the large fat-globules, it was observed that the small fat-globules also reacted in this manner to nitric acid, thus indicating that the pigment is soluble in both types of fat-globules. It should also be noted that when aphids were crushed under a cover-slip or killed by high temperatures (40° to 50°) or with ether fumes, the fat-globules usually became yellowish to a strong yellow in color before they solidified. It thus appears that the pigment is soluble in the fat-globules.

As has been previously noted, fat-globules from 24° aphids that have solidified do not liquefy until they have been heated to a temperature of approximately 65° . When, however, a few drops of concentrated nitric acid were added to the crushed aphid with its fat-globules already solid, the fat-globules, after a short interval, became liquid at room temperatures. Fat-globules that had been so treated remained liquid until a temperature of about 0°C. was reached and

the melting-point then coincided closely (within about 5° or 6°) with the solidification point.

Since the application of nitric acid apparently causes the crystallization or removal of the pigment from the fat-globules, then it seems likely that the lowering of the melting-point as described in the preceding paragraph is due to the removal or partial removal of the pigment from the fat-globules or, conversely, that the addition of pigment to the fat-globule in some way raises the melting-point. In other words, it appears that the melting- (or solidification) point of the fat-globule is altered by substances, probably the pigment, from the colored globules.

A further substantiation of this view is found in the following observations. The fat-globules of aphids were caused to solidify by two other methods than those which have been previously described. Aphids were subjected to a temperature of 45° for a few minutes and then allowed to cool. The pigmented globules were disrupted by this treatment and the fat-globules solidified at room temperatures. It might be objected that this solidification was caused by changes that accompany death, other than the breaking of the brown globules. But the fat-globules of aphids have been caused to solidify at room temperatures without death entering in, and without even the possibility of the intervention of oxidative changes such as might accompany fat solidification which occurs when aphids are crushed. This was done by merely tapping and rolling the aphid under a cover-slip with sufficient force to cause some of the fat-globules to coalesce. Coalescence indicated that the surrounding globules had been disrupted. When aphids so treated were left at room temperatures and examined an hour later, it was found that those globules which had coalesced had become solid at room temperatures, the other globules remaining liquid. These observations lead me to believe that the solidification of the fat-globules is dependent primarily upon the discharge of the contents of the brown globules and the influence of these substances upon the fat-globules.

The solidification of fat-globules as a result of exposing aphids to low temperatures as practiced in the fat-solidification experiments is also probably due to the breaking of the brown globules. Wingless aphids from the 24° chamber were subjected to temperatures of —4°, —5°, —6°, and —7° for a period of one hour. In each case the brown globules were all, or almost all, broken and the fat-globules solidified. At —7° the aphids were killed, but at —4° and —5° they remained alive. When aphids were exposed to —1°, —2°, or —3°, the brown globules would usually break if the exposure period was prolonged to four or five hours. Furthermore, aphids were subjected to temperatures of —1° and —2° for a period of one hour, after which there was no visible evidence of their brown globules having been broken, and the aphids were then returned to 24°. In aphids so treated the brown globules all, or almost all, broke during the succeeding twenty-four hours.

The evidence clearly shows that if an aphid that has been living at a temperature of 24° has its temperature reduced by from 25° to 28° or more, according to the time of exposure, all its brown globules break.

But, as may be readily observed, by crushing a normal untreated aphid and watching the breaking of its brown globules, some globules are more resistant than others to disturbing influences. Inasmuch as the solidification of the fat-globules was found to be dependent primarily upon the disruption of the brown globules, and since all the brown globules of 24° aphids were caused to break by exposures of —1° to —7°, there is every reason for believing that since some globules are less resistant than others, an exposure temperature of 7° causes a sufficient number of the brown globules to break to cause the fat-globules to solidify.

Briefly stated, it is believed that solidification of the fat-globules in aphids exposed to low temperatures is due to the influence of the temperature change being exerted primarily on the pigmented globules, and not directly on the fat-globules. This view is not only supported by the facts just given,

but it also affords a reasonable explanation of the wide gap that exists between the solidification temperature of the fat and its melting-point, as determined in the fat-solidification experiments. The physicochemical nature of the causes of fat solidification in these aphids is so obscure, however, that any explanation that is offered should be accepted with reserve until such time as the process may be more completely understood.

Conclusions concerning the brown globules

1) The brown globules are readily disrupted by disturbing influences, such as chemical or mechanical disturbances and temperature changes. 2) The brown globules are disrupted during the development of winged aphids, particularly during the processes involved in the last molt. No perceptible disruption occurs during the development of well-fed wingless aphids. 3) As a result of their disruption, the contents of the brown globules are discharged into the haemolymph, dissolved therein, and converted, at least in part, in winged aphids into some other material. 4) The pigment has chemical and biological affinities which suggest that it is an anthraquinone derivative. 5) It is believed that wing production may be dependent upon changes in the proportion or concentration of certain substances in the haemolymph as a result of the breaking of the brown globules. 6) The brown pigment is soluble in the fat-globules. 7) The contents of the brown globules when discharged into the surrounding media are believed to cause the large fat-globules to solidify. 8) The solidification of the fat-globules in aphids exposed to low temperatures is believed to be due to the influence of the temperature change being exerted primarily on the pigmented globules (causing them to break and discharge their contents into the surrounding media), and not directly on the fat-globules.

Discussion of the brown globules in their relation to the fat-globules

Three very interesting phases of the solidification of the fat-globules are still in need of consideration: 1) Why do the fat-globules of wingless aphids raised at a given habitat temperature show such a high degree of precision with relation to their solidification points? 2) Why do the fat-globules of wingless aphids raised at different habitat temperatures have different solidification temperatures? 3) Why do the fat-globules of winged aphids have solidification temperatures that are slightly below those of wingless aphids raised at the same temperature?

Since it appears that a sufficient number of brown globules are broken by subjecting 24° aphids to a temperature of 7° to cause the solidification of the fat-globules, it would seem that the high degree of precision which was so evident in the fat-solidification experiments merely indicates that a temperature change of about 17° for the 24° aphids is necessary to accomplish this result. Whether the temperature change exerts its influence directly on the brown globules or initiates activities in the surrounding haemolymph which result in the breaking of the brown globules is unknown. The latter alternative seems to be the more likely.

If this interpretation is correct, one would, of course, expect the fat-solidification point to differ with every habitat temperature. It may be noted, however, that in 28° aphids the spread between the habitat temperature and the fat-solidification temperature was not 17°, but 19°. For 18° and 16° aphids the spread was 15° and for the 12° aphids, 14°. This difference in spread may be due to the decrease in the quantity of brown pigment in aphids grown at higher temperatures, as was noted in the section on coloration, or the fat-globules may solidify at low temperatures as the result of the breakage of a smaller number of globules than are necessary to cause solidification at higher temperatures, or other factors, probably of a secondary nature, may enter in.

And why do the fat-globules of winged aphids have lower solidification temperatures than the fat-globules of wingless aphids reared at the same temperature? If the spread between habitat temperatures and fat-solidification temperatures represents the minimum temperature change necessary to cause the breaking of the weaker brown globules, then the spread must be greater in the case of winged aphids, because the less resistant globules have already been ruptured, or because the membranes of all the brown globules formed in winged aphids are tougher than in wingless aphids, or because the fat-globules themselves are different in the two forms. The first of the three suggestions is the most reasonable, especially in view of the fact that the brown globules are apparently degenerate or older forms of the green globules (Balbiani, '66; Uichanco, '24). If the fat-globules of the two forms are different, as they may be, it is more likely to be as a result of the breaking of the brown globules than by any other means, for the fat-globules are completely or almost completely encased in brown globules.

Since the less resistant brown globules are apparently absent in the nymphal as well as the adult stage of winged aphids, another piece of evidence is afforded which strongly indicates that wing production in aphids is dependent upon the breaking of the brown globules.

GENERAL DISCUSSION

Inasmuch as opportunities for chromosomal variations are at a minimum in a parthenogenetic line, and since the percentage of winged forms produced may be varied by changing environmental conditions, but particularly because the development of wings may be arrested by putting the aphids on a freshly germinated plant, it is believed that the variations which have been described as occurring in these aphids cannot be attributed to changes in the nature of the chromosomes. In other words, these variations are believed to be due to purely physiological rather than genetic conditions.

The occasional development of rudimentary ocelli in aphids that have no wings, the variations in the occurrence of sensoria, and the different degrees of cuticular marking—all of which have been shown to be strongly correlated with wing production—and finally the variations in the development of the wings themselves indicate that quantitative factors are involved.

Evidence has been presented which indicates that changes in the proportion or concentration of the materials in the haemolymph resulting from the rupture of the brown globules represents the quantitative factor that is involved.

A difficulty that remains is to determine whether the breaking of the brown globules causes wing development or whether wing development causes the breaking of the globules. It is obvious that, with the possible exception of temperature influences, if the brown globules are to be broken, it must be as the result of forces operating within the aphid. In other words, the breaking of the brown globules, ordinarily, is not likely to be the first link in the chain of events that leads to the production of winged forms. In my opinion, the fact that the fat-solidification temperatures of even the youngest aphids in which the development of wings is recognizable is markedly lower than for wingless aphids is strong evidence to show that the breaking of the brown globules is a link in the chain of causes, and not an end result. Furthermore, when wingless parents are starved, the brown globules are disrupted in the parent, while the offspring which receive their nourishment from the body fluids of the mother show a marked increase in wing production. In this case there can be no doubt of which is the cause and which the effect.

It still remains to examine the various phenomena that are associated with wing development from the point of view that has been presented.

If the foregoing suggestions are correct, one would expect winged parents to be less likely to produce winged offspring than are wingless parents (a relation which has been found

to exist) not only because a consumption of wing-producing material in the production of wings is to be presumed, but also because a reduction in the number of brown globules in the winged parent may actually be observed.

Also one would not expect starvation of winged parents to increase the percentage of winged offspring, for, quite obviously, starvation could not have an effect on winged aphids such as it has on the wingless form, since the brown globules are wholly or almost wholly absent in the winged form.

Since the disruption of the brown globules is readily effected by means of chemical change, one would expect this disruption to be increased as a result of periodic starvation of the wingless parents or overcrowding of their offspring. A marked reduction in the number of brown globules present in aphids that have been starved may be readily observed, especially after several days have elapsed, and, as has been previously shown, starvation of the wingless parent induces wing production very markedly.

It appears from the fat-solidification experiments that several days must elapse after aphids have been transferred to a new habitat temperature before any marked differences in the fat-solidification temperatures occur. If the interpretation of the causes of fat solidification that has been given is correct, this signifies that the less resistant brown globules remain intact during the first few days, and, furthermore, that this interval is followed by a protracted period during which the less resistant globules are gradually destroyed. If wing production is dependent upon the disruption of the brown globules, then there should be a short interval, corresponding with the period in which the less resistant globules are being destroyed, during which there was found to be no increase in wing production as a result of the temperature change. And also, since this interval is followed by a protracted period during which the less resistant globules are presumably being destroyed, an increase in wing production would be expected to occur throughout that period such as was found during the temperature experiments.

With reference to the effects of specific temperatures, it would appear from graph 1 that wing production may be associated with the rate of metabolism and that an interfering factor enters in at 18°. This interfering factor might be due to changes in protoplasmic viscosity, as previously suggested. Also, as has been noted, the proportion of brown and green globules present in aphids changes with changes of temperature. Packets of green globules are frequently found among the brown globules at 16° and below, but not at higher temperatures. It is believed that the balance which seems to exist between the two sets of globules, pigmented and fat, may in some way be responsible for the differences which specific temperatures appear to exert on wing production.

Of the three factors that affect wing production, starvation and type-of-parent are undoubtedly the strongest. It is difficult to compare the two. The temperature factor is undoubtedly weaker than the other two, but its true value is so difficult to determine because of the effects of even a short period of starvation or overcrowding on the results obtained, that it is almost impossible to judge just how significant the temperature factor is.

The colored illustrations in Buckton's well-illustrated work and a few observations that I have made on other species of aphids cause me to believe that color change is at least a common, if not a universal accompaniment of wing production in aphids. From this it seems to be probable that the processes that have been described as occurring in the grain aphid are processes which with minor modifications occur in all aphids.

The evidence indicating that wing production is dependent upon the concentration or proportion of certain substances in the haemolymph as effected by the disruption of the brown globules may be summed up as follows: 1) The brown globules are disrupted during the development of winged aphids, but not to any appreciable extent during the development of well-fed wingless aphids. 2) The brown pigment is usually found in the region of the developing wing buds in sufficient

concentration to give the haemolymph of that region a purplish tinge. 3) Winged parents are less likely to have winged offspring than are wingless parents, as would be expected, inasmuch as the brown globules are absent in the adult winged form. 4) Starvation of wingless parents causes a decrease in the number of brown globules and increases the percentage of wing production among the offspring. 5) Starvation of winged parents fails to increase wing production among the offspring, as would be expected, since no brown globules are present to be disrupted. 6) Overcrowding of the offspring of winged parents serves to increase wing production slightly, as would be expected, since the offspring develop brown globules of their own which may be disrupted. 7) Aphids changed from 24° to 34° – 36° for one hour and then returned to 24° show a five-fold increase in wing production and also show a decrease in the number of brown globules (the data upon which this statement is based were secured after this paper was written and does not appear in the preceding pages). 8) Small changes of temperature (24° to 16°) over longer periods of time appear to cause the brown globules to break and cause an increase in wing production. 9) The brown globules are unstable; their disruption causes the discharge of substances into the haemolymph; the instability of these globules might well be the basic cause of the instability which occurs in the aphid as a whole. 10) Variations in the degree of wing development and of the development of sensoria, ocelli, and cuticular markings, and the occurrence of intermediates indicates that a quantitative factor of some sort is involved. 11) The relation of fat-solidification temperatures to habitat temperatures, to overcrowding, and to the form of the aphid (winged or wingless) indicates that a fundamental relationship exists between wing production and fat solidification. The solidification of the fat-globules as well as wing production appears to be dependent upon the breaking of the brown globules.

SUMMARY

1. Approximately 20,000 grain aphids (*Rhopalosiphum prunifoliae* Fitch) were raised under controlled conditions to determine the effects of environmental influences on wing production. It was found that aphids raised at temperatures of 14° to 16° and 24° to 26° were more likely to be winged than those that were grown at temperatures of 12° or 18° to 20°. It was also found that when aphids that had been growing at a particular temperature for several generations were transferred to a new temperature, they produced a higher percentage of winged forms during the first two or three weeks than they did later on.

Winged parents were much less likely than wingless parents to produce winged offspring.

The offspring of wingless parents showed a very pronounced increase in wing production when the parents were removed from their food plants for a period of fifteen or sixteen hours or when the host plant was allowed to become heavily infested with aphids. The offspring of wingless parents reared on plants that were so weak from a lack of sunlight as to make it difficult to maintain the line were all wingless.

The offspring of winged parents showed a slight decrease in wing production when the parents were removed from the food plant for a period of fifteen or sixteen hours, but when the offspring of winged parents were allowed to overcrowd, a slight increase in wing production occurred.

Aphids were reared on plants growing in various salt solutions. Salts, as components of the food of aphids, were not found to have an effect on wing production; that is, it was not found possible to confirm the results of Clarke, Neils, and Shinji.

2. A study was made of various characters (ocelli, sensoria, cuticular markings, size, and coloration), changes in which are strongly correlated with wing production.

3. In the haemolymph of these aphids four kinds of globules were found. Two of the four globules are pigmented and are

largely responsible for the color of the aphid, the other two kinds of globules were found to be colorless lipoid globules.

The large fat- (lipoid) globules were caused to solidify by exposing the aphid to low temperatures for a period of one hour. It was found that the solidification temperature of the fat-globules was constant for aphids grown at a given habitat temperature and that with changes in the habitat temperature of aphids the fat-solidification temperature also changed.

The fat-solidification temperature of winged aphids was found to be several degrees lower than for wingless aphids raised at the same temperature.

When aphids were transferred from one temperature to another, the time required for the aphid to come into equilibrium with its new thermal environment as determined by changes in the fat-solidification temperatures varied from one week to more than two weeks, the time being considerably shortened when overcrowding was permitted and when the offspring rather than the original aphids were tested. It was also shown that the temperature at which an aphid lives affects the fat solidification temperature as the result of a direct effect of the temperature on the aphid, and not because of the effect of the temperature on the plant.

The fat-globules solidified as a result of exposure to low temperatures (7° to -3°), but the solidified fat-globule of 24° aphids did not melt at a temperature lower than approximately 65°C . It was shown that the brown pigment from the pigmented globules is soluble in the fat-globules and that the contents of the brown globules caused the fat-globules to solidify at higher temperatures than they otherwise would.

The brown globules possess a delicate confining membrane which is readily broken as a result of chemical, mechanical, and thermal disturbances. Evidence was presented which strongly indicates that the solidification of the fat-globules by exposure to low temperatures is due not to the effect of the temperature change on the fat-globule directly, but rather to the effects of the temperature change on the brown globule. It is believed that a certain minimum temperature change is

required to cause the disruption of the less resistant brown globules and that the material which is discharged from the broken globules causes the fat to solidify.

From the literature and from my observations it seems that the brown pigment may be an anthraquinone derivative which is of a very unstable nature.

It is believed that wing production in the grain aphid is dependent upon changes in the proportion or concentration of certain materials in the haemolymph as brought to pass by the rupture of the brown globules. A considerable amount of evidence, which is summed up at the end of the general discussion, is presented to that effect.

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